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REMOVING COMPLEX GROWTH MEDIA FROM MS2 BACTERIOPHAGE CULTURES

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Purification and concentration of viruses from the background material are required whatever the subsequent analysis methods used. For the analysis of viruses, it is essential, and detection methods depend on this solution. This report demonstrates a methodology for removing growth media from a virus preparation. A sample of MS2 was purified using a new ultrafiltration (UF) technique with hollow fibers. A typical MS2 virus sample with a nominal stated concentration of 1.4×10^{12} plaque forming units per ml (pfu/ml) in the original growth media was used to demonstrate this method. After UF, the growth media was removed and the viruses counted using the Integrated Virus Detection System (IVDS) instrument.

This report further describes the use of this ultrafiltration procedure to remove other impurities, such as cesium chloride and albumin, from solutions containing a purified solution of MS2 bacteriophage. These solutions were also analyzed using the IVDS instrument.

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Preface

The work described in this report was performed as part of a Defense Advanced Research Projects Agency (DARPA), Defense Sciences Office project. This work was started in March 1998 and was completed in September 1998.

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Removing Complex Growth Media From MS2 Bacteriophage Cultures

1. Introduction

There are many inherent challenges to virus detection and analysis. One of the more important is purification and concentration from the background material. This is required whatever the detection method to be used in subsequent steps. The background loading which may contain growth media, salts, proteins and other material all make this issue a challenge. It is possible that there is little purpose to even considering detection of viruses until these steps are resolved. One step is the removal of growth media and other impurities such as salts and proteins.

A sample of MS2 bacteriophage was received from the Life Sciences Division at Dugway Proving Ground (DPG). This sample was 500 ml of as grown MS2 bacteriophage, complete with growth media, at a virus concentration of 1.4×10^{12} pfu/ml. The growth media was comprised of L-B broth, 10 g Tryptone, 10 parts NaCl and 5 parts yeast extract. The MS2 solution was a dark yellow color and is clear. The sample was from Lot #98251.

The MS2 sample was analyzed using either the IVDS instrument[1] or more directly the Gas-phase Electrophoretic Mobility Molecular Analyzer (GEMMA) detector and ultrafiltration module are two stages of the IVDS instrument. The GEMMA detector consists of an electrospray unit to inject samples into the detector, a Differential Mobility Analyzer and a Condensate Particle Counter. A complete description of the IVDS system, including the GEMMA detector, can be found in the report *Virus Detection: Limits and Strategies.*[2]

Several solutions were prepared to explore the ability of the ultrafiltration apparatus to remove contaminates and retain viruses of interest in solution. A sample of albumin, from chicken egg, was prepared at a concentration of 0.02%, by weight, in an ammonium acetate (0.02M) buffer. To this solution was added MS2 bacteriophage to a concentration of 3×10^{11} pfu/ml. A second solution was prepared containing 2.5% cesium chloride (CsCl), by weight, also in the ammonium acetate buffer. . To this solution was added MS2 bacteriophage to a concentration of 5×10^{11} pfu/ml. The MS2 bacteriophage, in both cases, was a highly purified sample obtained from DPG Life Sciences Division (Lot #98110).

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[1] Patent Pending on IVDS technology.

[2] Wick, C.H., Yeh, H.R., Carlon, H.R., and Anderson, D., *Virus Detection: Limits and Strategies*, ERDEC-TR-453, December 1997.

2. Laboratory Testing of MS2 Bacteriophage

2.1 Results of MS2 plus Growth Media

The mixed MS2 sample, with $1.4x10^{12}$ pfu/ml was analyzed using the GEMMA virus detector. The sample was placed neat into the GEMMA analyzer and the results are shown in Figure 1. The growth media, with the MS2 Bacteriophage in solution, produces a graph that displays a very broad, nondescript peak across the area of interest of 24-26 nm. The size range of 24-26 nm is the expected size for a MS2 bacteriophage, as shown in Figure 2 in a micrograph by Dr. Hans Ackermann[3]. It is not apparent from the as received sample analysis if the sample actually contains MS2 in solution. The solution required removal of the growth media before any meaningful results could be obtained.

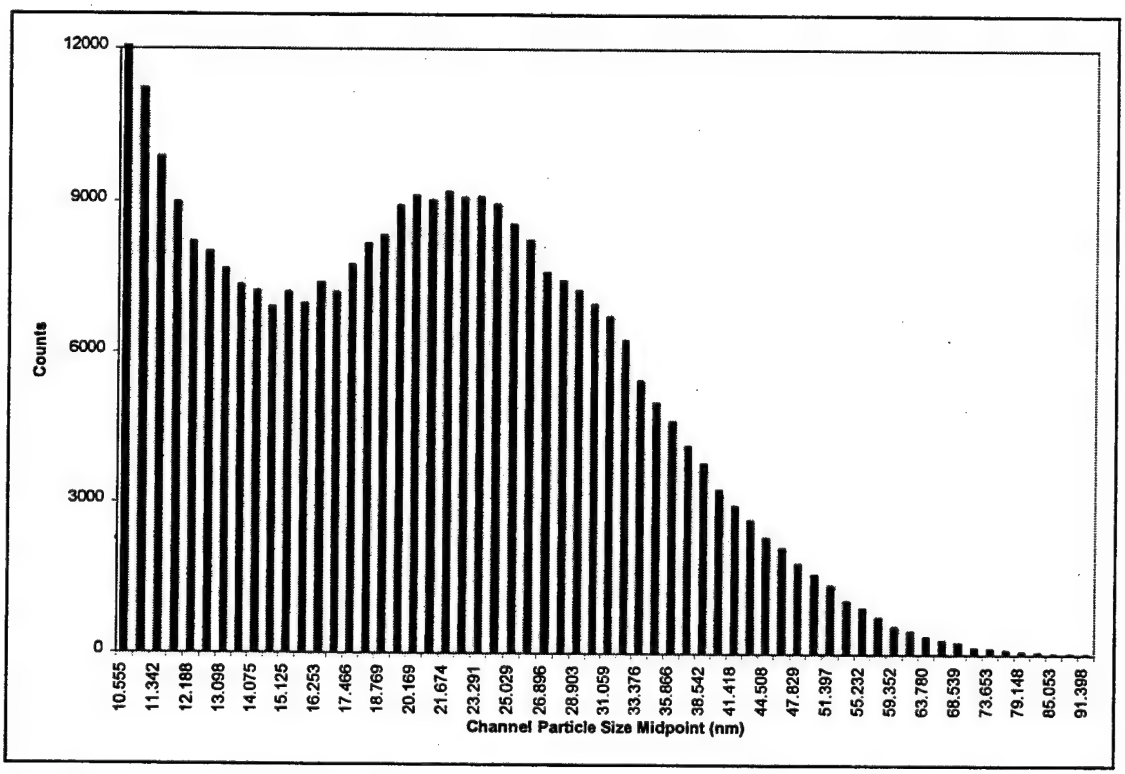

Figure 1 GEMMA Analysis of MS2 Bacteriophage plus Growth Media, DPG Lot #98251

[3] Micrograph located at http://life.anu.edu.au/viruses/welcom.htm.

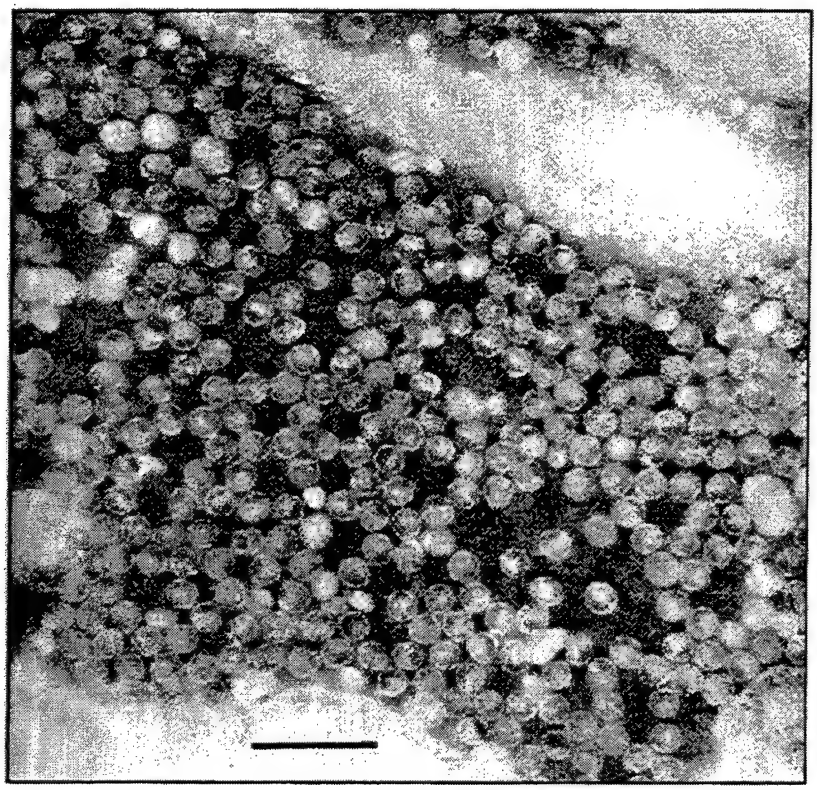

Figure 2 Micrograph of MS2 Bacteriophage (bar represents 100 nm)

2.1.1 Ultrafiltration of MS2 plus Growth Media

The virus plus growth media sample was purified and concentrated using an ultrafiltration (UF) process. The UF module, shown in Figure 3, is used for processing and retaining a virus species for further study. The UF stage is a hollow fiber-based tangential or cross flow filtration system. These filtration systems operate by pumping the feed stream through the hollow fiber, as shown in Figures 4 and 5. As the solution passes through the fiber, the sweeping action of the flow helps to prevent clogging of the fiber. A pressure differential forces the filtrate through the fiber, while the virus feed stream is purified and concentrated. There are available a wide range of pore sizes for the fibers. This filtration technique can reduce volumes from over 5 ml to 0.2 ml.

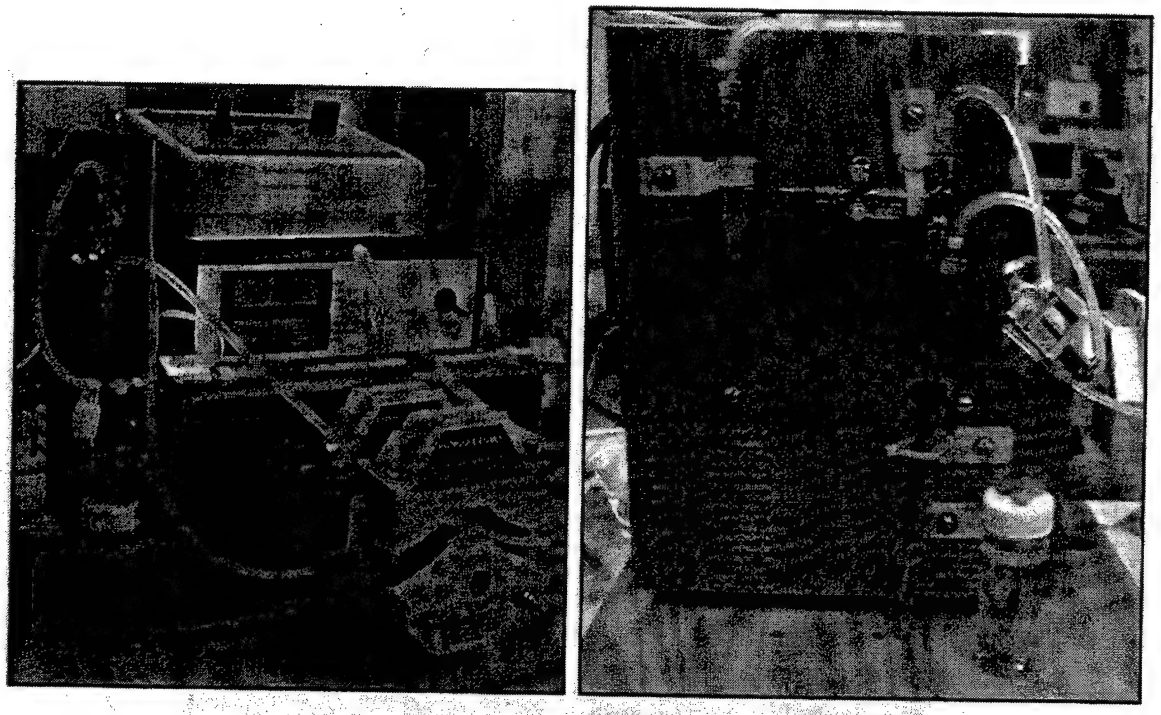

Figure 3 Front and Side Views of UF Module 1

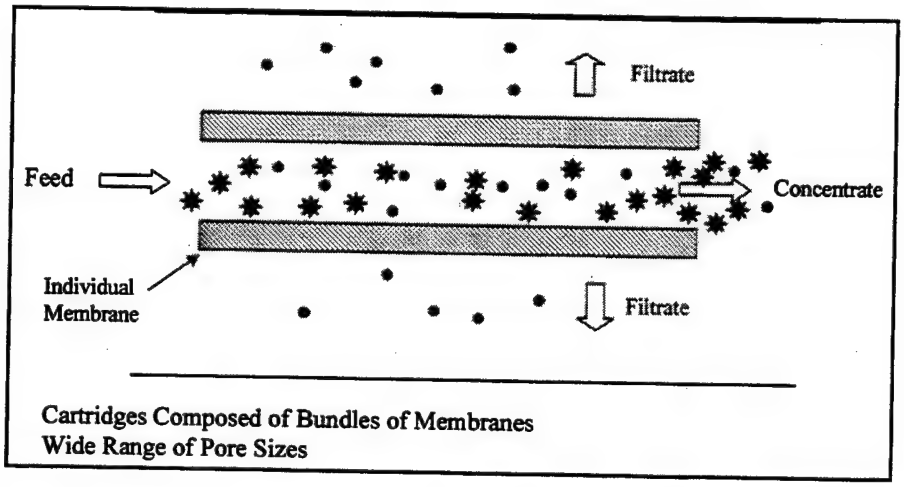

Figure 4 Graphical Representation of Cross Flow Filtration - Individual Fiber

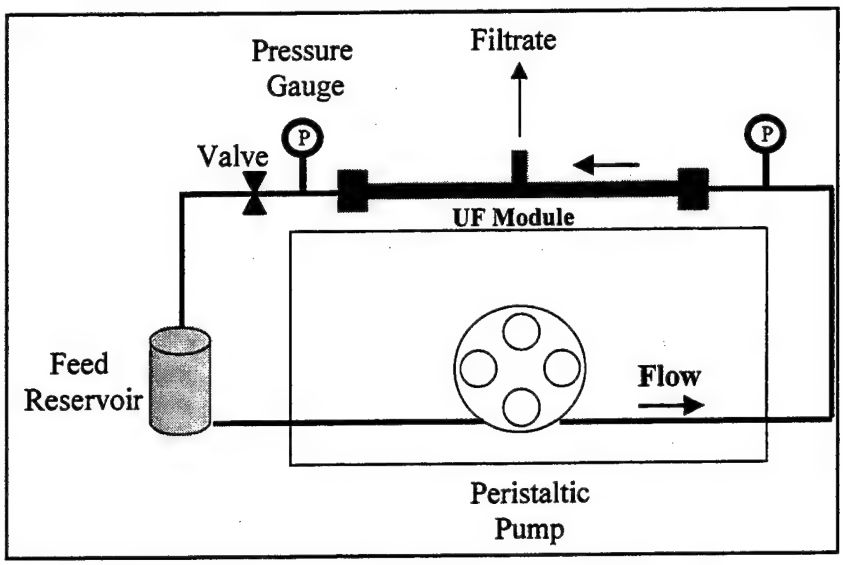

Figure 5 Flow Diagram of Ultrafiltration Apparatus Utilizing the Cross Flow Filtration Process

A sample of the DPG MS2 with growth media was then processed through the ultrafiltration apparatus. The parameters for ultrafiltration are listed in Table 1.

Table 1 UF Parameters for MS2 in Growth Media

Sample volume-initial	3 ml
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	50 ml
Sample volume-final	2 ml
MWCO of module	500K

By continually washing the sample volume with ammonium acetate buffer (the working fluid of the GEMMA analyzer), the UF apparatus will allow the removal of ions, proteins and all other material that is smaller than the 500K molecular weight cut-off (MWCO) of the cross flow filter. The MS2 bacteriophage will be retained in the circulating solution and continue to be purified by the process. As the 500K MWCO filter will effectively retain the MS2, the total wash volume can be significantly larger than the initial sample volume. The ultrafiltration of this sample was completed in less than 10 minutes.

2.1.2 Counting of MS2 after Removing Growth Media

After ultrafiltration, the sample was then analyzed in the GEMMA. The results are shown in Figure 6. The graph shows that most if not all of the growth media has been removed and replaced with the ammonium acetate buffer, which is virtually invisible to the GEMMA analyzer. The remaining solution from the ultrafiltration apparatus is very pure and can be used for further experimentation. The numerical results, as shown in Table 2, show a very low count rate, essentially a background level, outside the area of peaks for MS2. At this level of purification and concentration, the original 500 ml of as received MS2 with growth media, could yield over 300 ml of very pure, high concentration MS2 bacteriophage in ammonium acetate.

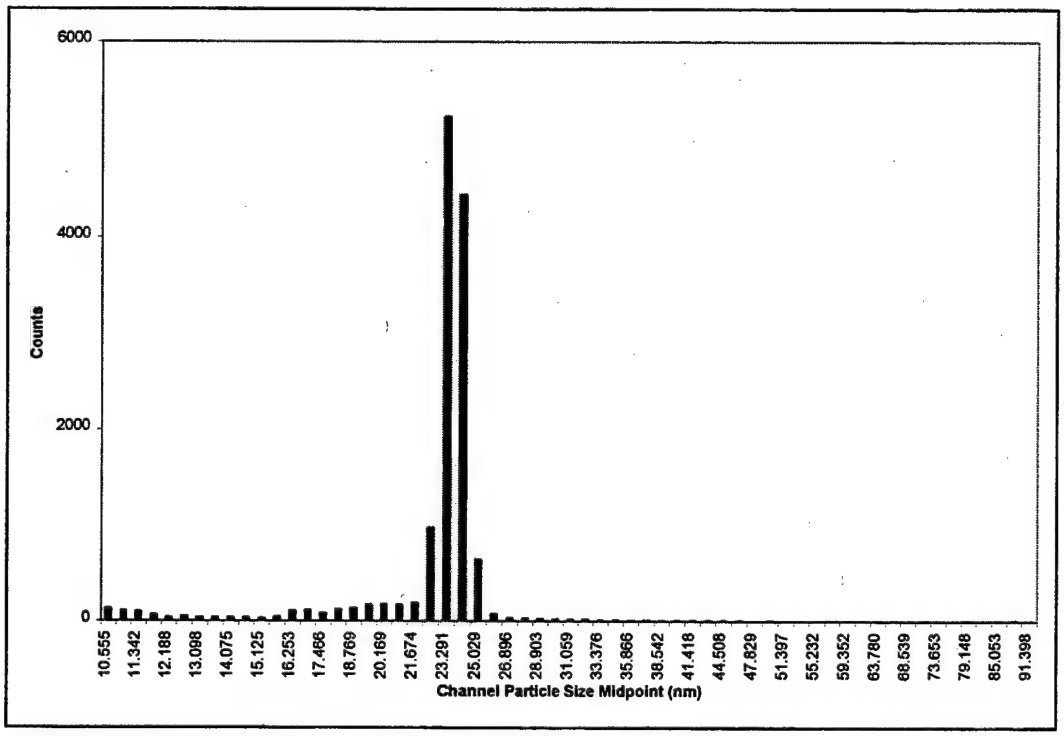

Figure 6 GEMMA Analysis of MS2 (Growth Media Sample)-Ultrafiltration Processed

Table 2 GEMMA Counts for MS2 Bacteriophage

Channel Midpoint Diameter (nm)	Counts	Channel Midpoint Diameter (nm)	Counts
10.5545	128.2	32.1968	17.5
10.9411	105.7	33.3762	8.5
11.3419	97.7	34.5989	10.5
11.7574	64.3	35.8664	7.5
12.1881	37.3	37.1803	6.5
12.6346	50.8	38.5423	2.5
13.0975	34.2	39.9542	3
13.5773	36.8	41.4178	6.8
14.0746	39.5	42.9351	2.2
14.5902	34.6	44.5079	5
15.1247	28	46.1384	2
15.6788	41.5	47.8286	1
16.2531	102.1	49.5807	2
16.8485	110.8	51.397	1
17.4658	80.3	53.2798	0
18.1056	120.7	55.2316	1
18.7688	129.4	57.2549	0
19.4564	167	59.3523	0
20.1691	175.2	61.5265	1
20.908	168.6	63.7804	1
21.6739	192.2	66.1169	0
22.4679	973.1	68.539	1
23.291	5228.2	71.0497	1
24.1442	4429.7	73.6525	1
25.0287	639.9	76.3506	0
25.9455	73.6	79.1476	1
26.896	31.4	82.047	0
27.8813	25.6	85.0526	0
28.9026	22	88.1683	2
29.9614	19.5	91.3982	0
31.059	16.2		

2.2 Results of MS2 plus Albumin

The sample of 0.02% albumin in ammonium acetate, with the addition of $3x10^{11}$ pfu/ml of MS2 bacteriophage, was analyzed neat in the GEMMA virus detector. As shown in Figure 7, the MS2 peak is centered around 24 nm. The albumin in the sample is displayed as a very broad peak starting below 10 nm and extending to 20 nm.

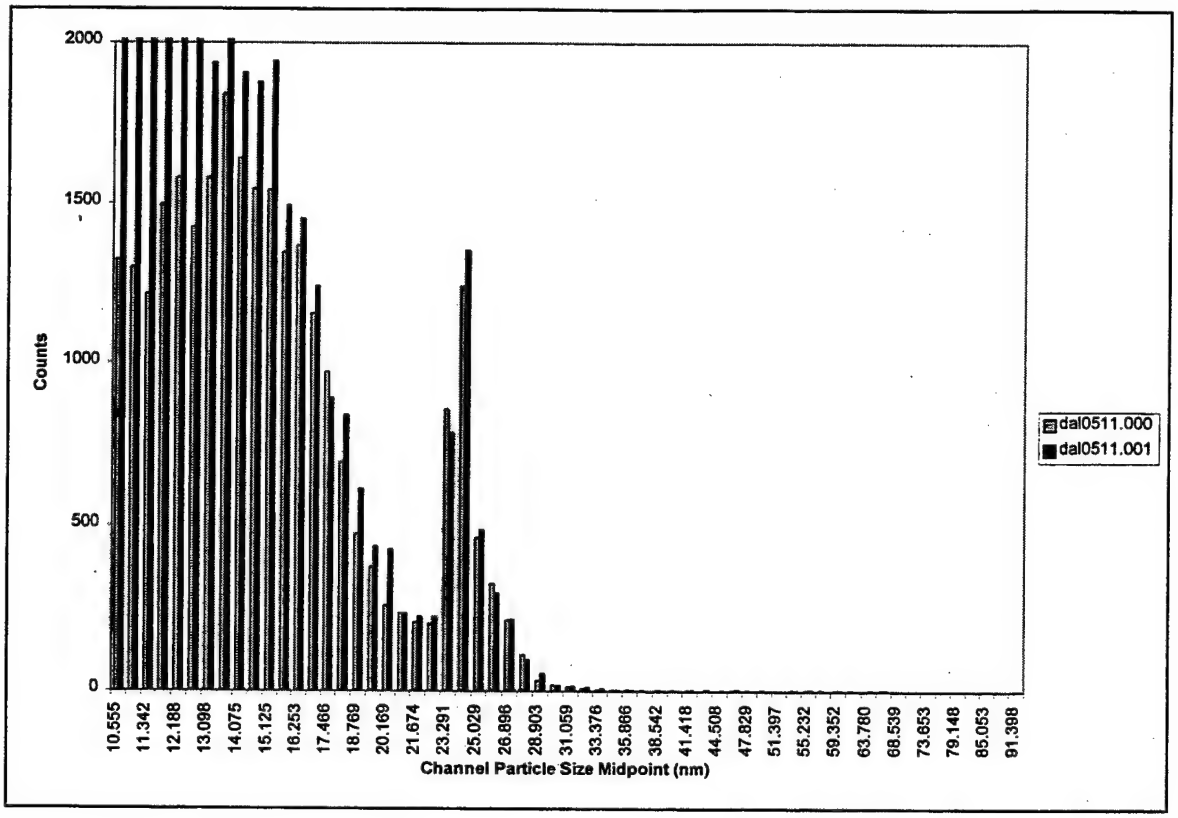

Figure 7 GEMMA Analysis of Albumin (0.02%) in Ammonium Acetate with MS2 ($3x10^{11}$ pfu/ml)

2.2.1 Ultrafiltration of MS2 Plus Albumin

The sample of albumin plus MS2 was then processed through the ultrafiltration apparatus. The parameters for the ultrafiltration are shown in Table 3.

Table 3 UF Parameters for Albumin plus MS2

Sample volume-initial	1 ml
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	40 ml
Sample volume-final	0.4 ml
MWCO of module	500K

2.2.2 Counting of MS2 After Removing Albumin

After processing in the ultrafiltration apparatus, the sample was examined in the GEMMA virus detector. As shown in Figure 8, the only peak in evidence is centered on 24 nm. The large peak between 10 and 20 nm was completely removed. The processing of the sample

through the ultrafiltration apparatus completely removed the albumin protein, while the MS2 bacteriophage was retained.

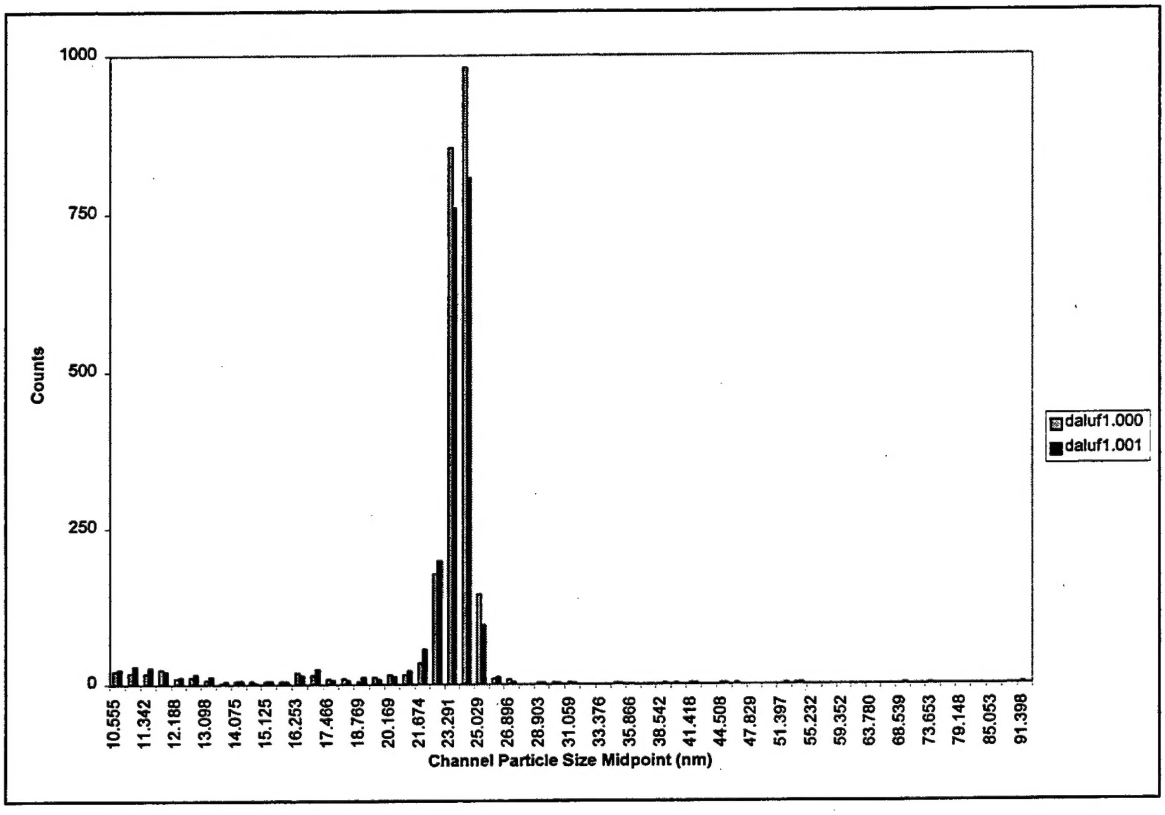

Figure 8 GEMMA Analysis of Albumin plus MS2 Bacteriophage-Ultrafiltration Processed

2.3 Results of MS2 plus Cesium Chloride

The sample of 2.5% CsCl, by weight, in ammonium acetate, with the addition of $5x10^{11}$ pfu/ml of MS2 bacteriophage, was analyzed neat in the GEMMA virus detector. As shown in Figure 9, the MS2 peak is centered around 24 nm. The CsCl in the sample is displayed as a very broad peak starting below 10 nm and extending to over 20 nm. Any higher concentrations of CsCl would start to obscure the MS2 peak position.

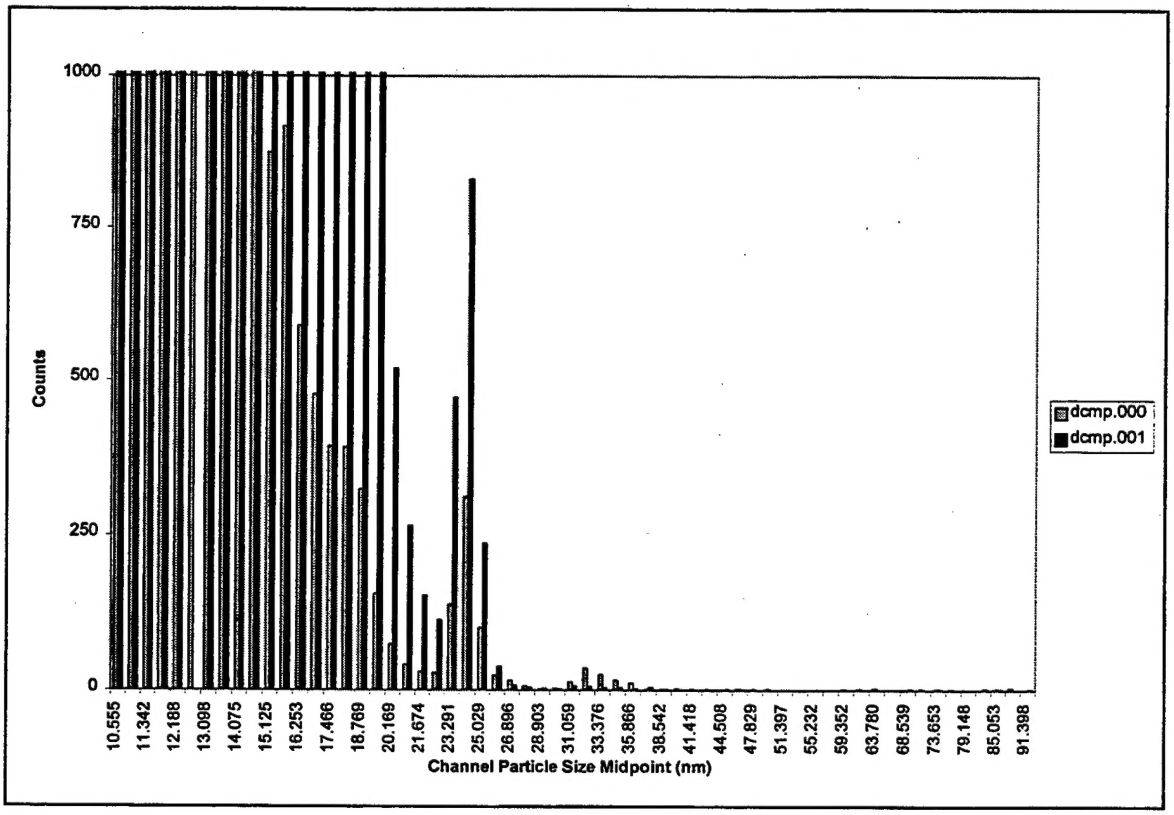

Figure 9 GEMMA Analysis of Cesium Chloride (2.5%) plus MS2 ($5x10^{11}$ pfu/ml)

2.3.1 Ultrafiltration of MS2 plus Cesium Chloride

The sample of CsCl plus MS2 was then processed through the ultrafiltration apparatus. The parameters for the ultrafiltration are shown in Table 4.

Table 4 UF Parameters for CsCl plus MS2

Sample volume-initial	1 ml
Pump speed	2
Transducer pressure	15 psig
Total buffer wash volume	30 ml
Sample volume-final	0.5 ml
MWCO of module	500K

2.3.2 Counting of MS2 after Removing Cesium Chloride

After processing in the ultrafiltration apparatus, the sample was examined in the GEMMA virus detector. As shown in Figure 10, the MS2 peak is shown centered on 24 nm. The large peak between 10 and 22 nm was significantly removed. There was a small remnant of the

CsCl peak in the processed sample due to the smaller amount of buffer wash volume in this cycle. To completely remove the CsCl, the ultrafiltration process would only need to be continued with further washing until all of the salt was replaced with buffer solution. The processing of this sample through the ultrafiltration apparatus also retained the MS2 bacteriophage.

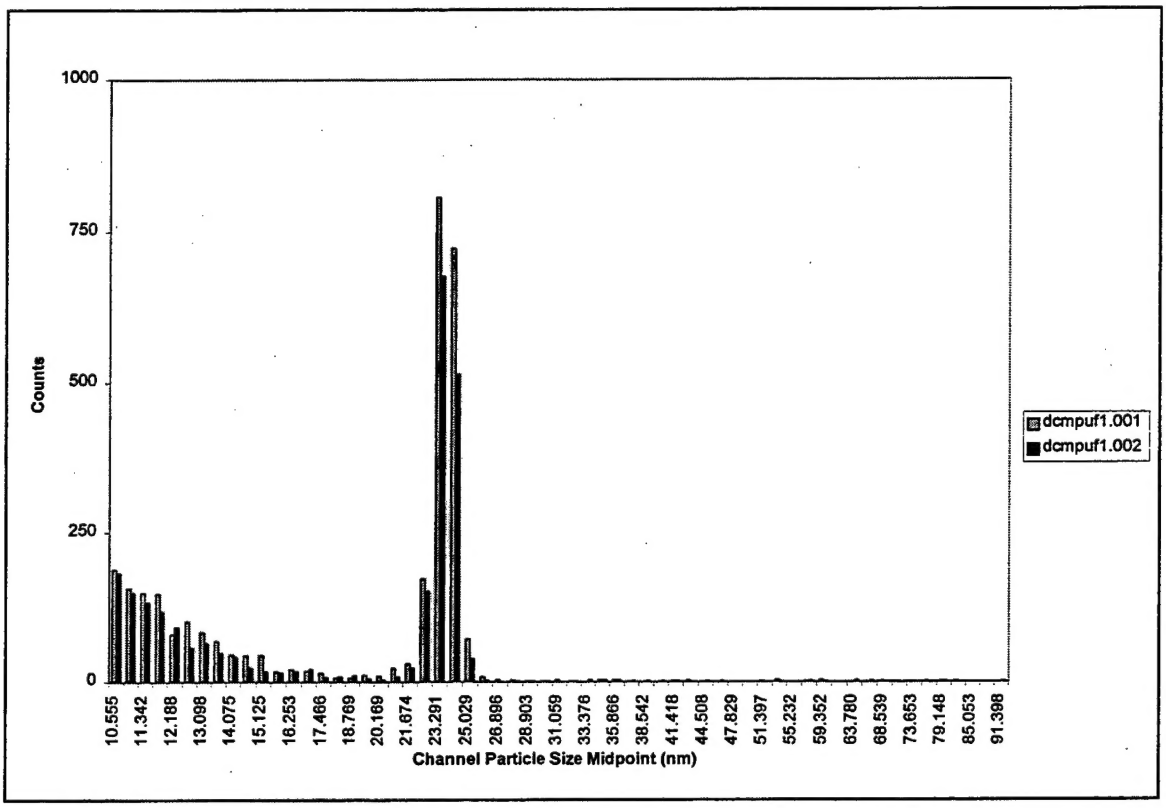

Figure 10 GEMMA Analysis of Cesium Chloride plus MS2-Ultrafiltration Processed

3. Analysis

The ultrafiltration apparatus was very effective in removing the growth media from the as received solution of MS2 bacteriophage. The addition of approximately ten times the amount of starting solution with ammonium acetate buffer (3 ml vs. 50 ml respectively) allowed the efficient replacement of the growth media with the buffer solution. The background of the GEMMA scan of the ultrafiltration-processed solution was very low due to the low detection of ammonium acetate. In addition, the ultrafiltration process for comparable volumes can be completed in approximately 10 minutes.

The addition of other contaminating materials in a virus solution can also be successfully removed from solution while retaining the virus. The albumin protein was almost completely removed from the MS2 containing solution by ultrafiltration. The adjustment (if necessary) of

the pore size of the ultrafiltration modules allows for great flexibility in the processing of solutions.

The CsCl solution appeared to require further washing to completely remove the salt from the virus containing solution. From the few tests to date, it appears that the wash volume for the removal of CsCl in the ultrafiltration apparatus requires the initial sample volume to be washed with approximately 40-50 times the volume of buffer solution, for certain impurities, to completely remove those impurities.

4. Conclusions

The sample of MS2 bacteriophage containing original growth media was purified by ultra-filtration. The growth media was removed by this process and the MS2 bacteriophage retained. The ultrafiltration module was equipped with a 500K molecular weight cut-off cross flow filter, which effectively retained the bacteriophage while allowing the removal of the growth media. The remaining solution of bacteriophage can now be further characterized and the process used as a purification step for further analysis. This technique should be compatible for removing similar material from solutions of other viruses.

Samples of laboratory produced solutions, specifically albumin protein and CsCl solutions with MS2 bacteriophage were successfully processed in the ultrafiltration apparatus which removed these typical contaminates and retained MS2.